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Validation and application of micro flow liquid chromatography–tandem mass spectrometry for the determination of pesticide residues in fruit jams

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ABSTRACT

In this study, a very sensitive method was validated to determine pesticides residues in fruit jams using micro flow liquid chromatography–tandem mass spectrometry (μLC–MS/MS). A slurry of the fruit jams and water was prepared to yield homogeneous samples. Because of the high sensitivity achieved with the μLC-MS/MS equipment and to minimize matrix effects, the QuEChERS extracts were diluted 30-fold before the analysis. The validation was performed analyzing spiked samples at 9 and 45 μg kg^{-1} ($n=5$). The method met validation criteria of 70–120% recovery and RSD ≤ 20% for 92% of the 107 pesticides evaluated. The reporting limit (RL) was 9 and 45 μg kg^{-1} for respectively 66% and 26% of the analytes, 5% of the compounds did not fulfill the requirements for validation and 3% were not detected at the studied concentrations. The validated method was applied to the analysis of 51 different fruit jam samples from Brazil and Spain and pesticide residues were detected in 41 samples, 26 of which contained at least one pesticide at concentration > 10 μg kg^{-1}.

1. Introduction

Historically, jams originated as an early effort to preserve fruit for consumption during the fruit off-season. It is an intermediate moisture food prepared by boiling fruit pulp with sugar, pectin, acid and other ingredients (preservatives, coloring and flavoring substances) until obtaining a reasonably thick consistency [1] and a final water content of 32–34% [2]. According to the Document SANCO 12571/2013 [3] fruit jams are classified as high sugar and low water content commodities, together with honey and dried fruits and these characters of the fruit jams can represent a challenge in pesticide determination in such matrices [4].

Pesticides are chemical substances applied to crops at various stages of cultivation and during the post-harvest storage of crops. The use of pesticides is intended to prevent the destruction of food crops by controlling agricultural pests or unwanted plants and to improve plant quality [5]. In Brazil, one of the world's major food producers, over 90% of farmers rely on pesticide use and the country has ranked first in pesticide use worldwide in recent years, with over 673 million tons applied in 2008 [6].

Although pesticides help to control agricultural pests and organisms harming human activities, they may present a risk for human health. In the European Union (EU), the evaluation of plant protection products and the monitoring of pesticide residues in food are harmonized through Regulation EC No 1107/2009 and Regulation EC No 396/2005 [7]. In Brazil, the basis for pesticide regulation was set by Federal Law No. 7802, enacted in 1989, and later by Acts 4074/2002 and 5981/2006. Two monitoring programs for pesticide residues are currently in place in Brazil that aim to evaluate compliance with national MRLs: the Program on Pesticide Residue Analysis in Food (PARA), coordinated by the National Health Surveillance Agency (ANVISA), which aims to analyze fruits and vegetables, and the National Residue and Contaminant Control Program (PNCRC), coordinated by the Ministry of Agriculture, Livestock and Food Supplies (MAPA), that intent to control animal products, fruit and vegetables products [6,8,9]. None of these programs aims to evaluate processed food products, like e.g. fruit jams, juices or tomato purees, for pesticide residues.

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Liquid chromatography (LC) is used in many analytical applications worldwide and is commonly coupled to mass spectrometry (MS) to detect, to identify and to monitor compounds [10]. The development of miniaturized LC started by the mid-1970s but the first commercially available micro (μ) LC system was announced in late 1975 [11]. μLC typically uses columns with an internal diameter (I.D.) of 0.5 to 1 mm [12], lower mobile phase flow rates (1 to 40 μL min^{-1}) and present numerous advantages compared to conventional LC [13] like the ability to work with smaller sample sizes, lower volumetric flow-rates and the improvement in detection performance with the use of concentration-sensitive detectors as a result of the reduced chromatographic dilution [12,14].

It is considered that the increase of detection sensitivity in tubing with a small inner diameter is due to reduced axial sample band diffusion [15,16]. The following rationale suggests the selection of capillary LC. During chromatographic separation, the dilution (D) of an injected sample ($D=C_{end}/C_{inj}$, where C_{end} is the concentration after chromatography and C_{inj} is the concentration injected) is given by

$$D = \frac{\in \pi r^2 (1+k)(2LH)^{\frac{1}{2}}}{V_{inj}}$$

where $\in$ is the column porosity, r is the column radius, k is the retention factor, L is the column length, H is the plate height, and V_{inj} is the injection volume. If conditions are otherwise equal, D is in direct proportion to the square of column radius. When compared to conventional LC, μLC increases the signal-to-noise ratio (S/N) drastically when electrospray ionization (ESI) coupled to MS/MS is employed [14,17]. For example, from the previous equation it can be calculated that this will result in a 235-fold increase in peak height and mass sensitivity for a reduction in the diameter of a column from 4.6 mm to 300 μm I.D. [14], when all the other parameters are kept constant.

ESI is a soft ionization technique and these techniques perform considerably better if most of the eluate solvents are removed before the ionization process takes place. μLC delivers sharper and narrower solute bands to the interface nebulizer using a minimal amount of an appropriate solvent mixture. Consequently, smaller droplets are generated carrying less solvent to evaporate. The solute, which is distributed among a larger number of lower mass particles, is rapidly vaporized into the ion source minimizing thermal decomposition [18].

Due to the increase of pesticides applied in agriculture, their potential accumulation in both the environment and foods and their toxicities to humans a stricter control of residues in food commodities should be applied. Considering the decrease of the maximum residue limits (MRL) in most countries and continuous further prohibition of older, more harmful pesticides there is a need for sensitive multi-residue methods for monitoring and enforcement of the residues that may be present in food [19] including for processed food crops like fruit jams. The goal of this work was to develop and validate a selective, robust and highly sensitive μLC–MS/MS method to determine pesticides residues in fruit jams and later apply it to the analysis of samples to verify the existence of pesticides in these commodities.

2. Material and methods

2.1. Reagents and materials

Acetonitrile, HPLC grade (99.9%), formic acid, analytical grade (≥96.0%) and magnesium sulfate (98.0%) were purchased from Sigma Aldrich (Steinheim, Germany). Water, Optima®, HPLC grade was supplied by Fisher Scientific (New Jersey, USA). Sodium chloride (99.0%) was obtained from J.T. Baker (Deventer, Netherlands). Ethyl acetate, HPLC grade, sodium citrate tribasic dehydrate (99.0%) and disodium hydrogencitratesesquihydrate (99.0%) from Fluka (Steinheim, Germany). C_{18} (40 μm) was from Varian (Middelburg, The Netherlands) and Primary-Secondary Amine (PSA) Bond-Elut from Supelco (Bellefonte, USA). Pesticides standards (purity > 98.0%) were obtained from Dr. Ehrenstorfer (Augsburg, Germany), from Riedel-de Haën (Seelze, Germany) and from Sigma–Aldrich (Steinheim, Germany) and stored in a freezer at −30 °C.

2.2. Pesticide standard solutions

Individual pesticide standard stock solutions were prepared in acetonitrile and in ethyl acetate, at 1000 − 2000 mg L^{-1} and stored in amber screw-capped glass vials at −20 °C. A standard mixture solution of 107 pesticides was prepared in acetonitrile at the concentration of 1000 μg L^{-1}. This solution was used as spiking solution for recovery experiments and also to prepare the standard solutions in matrix (matrix-matched calibration standards) and organic solvent to obtain the calibration curves, by dilution with blank fruit jam extract or acetonitrile, respectively. The standards in blank matrix extract were used for the determination of the matrix effect and also for the recovery calculations.

2.3. Instrumentation

The chromatographic system consisted of an Eksigent ekspert™ μLC 200 (Eksigent, Redwood City, CA, USA) integrated to a hybrid quadrupole/linear ion trap mass spectrometer (QTRAP® 4500 MS/MS, AB Sciex Instruments, Foster City, CA, USA). Chromatographic separations were performed using an Halo C_{18} column 50 × 0.5 mm I.D. and 2.7 μm particle size (Eksigent, AB Sciex Instruments, Foster City, CA, USA) held at 30 °C by a column heater. The mobile phases consisted of water with 0.1% formic acid (mobile phase A) and acetonitrile with 0.1% formic acid (mobile phase B). The injection volume was 3 μL, the flow rate used was kept constant at 30 μL min^{-1} and the gradient program in positive mode was set as follows: 20% B (initial conditions) was kept constant for 1 min followed by a linear gradient up to 98% B in 9 min, after which the mobile phase composition was maintained at 98% A for 3 min, the re-equilibration time was 1 min and the total run time was 14 min.

The QTRAP® 4500 MS/MS system was equipped with an ESI source with μ-Flow electrode (50 mm I.D.), operating in positive and negative ionization mode, there was applied scheduled multiple reaction monitoring (sMRM) software features. The Turbo Ion Spray source settings were ion spray voltage, 5000 V; temperature, 400 °C; curtain gas flow, 20 L min^{-1}; collision gas, medium; and ion source gas (nebulizer gas and turbo gas), at a pressure of 30 psi. Nitrogen was used as the nebulizer gas, turbo gas, curtain gas and collision gas. The data were acquired and processed with the Analyst software version 1.6.2.

2.4. Selected μLC–MS/MS parameters

To optimize the mass spectrometer parameters an individual solution of each target compound was prepared in methanol at the concentration of 0.1 μg L^{-1}. Using flow injection analysis of these solutions, it was possible to optimize all the parameters including declustering potential, entrance potential, collision energy and collision cell exit potential for each single compound. The system was operated in a sMRM mode, through the acquisition of single reaction monitoring (SRM) transitions for each analyte with resolution set to Unit at the first and third quadrupoles. The time window was from 30 s for each SRM transition. According to Lozano et al. [20] the sMRM enables optimized cycle time and maximized dwell times to be used during acquisition to provide higher multiplexing with good analytical precision.

Table 1
Chromatographic parameters of the compounds determined by μLC–MS/MS. Retention time (t_R), declustering potential (DP) and collision energy (CE).

Compound	t_R (min)	DP (V)	Precursor ion (*m/z*)	Product ion (*m/z*)—SRM1	CE 1 (V)	Product ion (*m/z*) —SRM2	CE 2 (V)	Ionization mode
Acephate	0.44	50	184.0	94.9	30	143.0	11	ESI (+)
Acetamiprid	1.02	77	223.0	126.0	30	56.0	28	ESI (+)
Azinphos-methyl	4.15	55	318.0	132.1	21	261.0	8	ESI (+)
Azoxystrobin	4.48	57	404.0	372.0	20	344.0	33	ESI (+)
Bitertanol	4.94	60	338.0	269.1	14	99.1	24	ESI (+)
Boscalid	4.55	105	343.0	307.0	27	140.0	24	ESI (+)
Bromuconazole	4.24	100	378.0	159.0	40	70.0	59	ESI (+)
Bupirimate	3.57	100	317.0	166.1	32	272.1	27	ESI (+)
Buprofezin	4.60	62	306.0	201.0	17	116.0	23	ESI (+)
Carbaryl	2.92	56	202.0	145.0	13	127.0	37	ESI (+)
Carbendazim	0.45	113	192.0	160.0	26	132.0	43	ESI (+)
Carbofuran	2.69	56	222.0	165.0	16	123.0	26	ESI (+)
Chlorpyrifos	6.92	66	352.0	200.0	28	125.0	27	ESI (+)
Chlorpyrifos methyl	5.96	65	321.8	125.0	26	289.7	23	ESI (+)
Cyproconazole	4.16	85	292.0	125.0	48	139.0	32	ESI (+)
Cyprodinil	3.22	130	225.6	93.1	48	108.1	33	ESI (+)
Diazinon	5.60	102	304.8	169.2	29	153.1	28	ESI (+)
Diclorvos	2.33	77	220.9	109.0	24	127.0	24	ESI (+)
Diclorvos-d_6 (I.S.)	2.18	66	226.8	115.0	25	83.0	37	ESI (+)
Dicrotophos	0.49	63	238.0	112.0	17	193.1	13	ESI (+)
Diethofencarb	4.13	61	268.0	226.1	13	180.1	25	ESI (+)
Difenoconazole	5.44	105	406.0	251.0	35	337.0	25	ESI (+)
Dimethoate	0.94	50	230.0	199.0	12	171.0	19	ESI (+)
Dimethoate-d_6 (I.S.)	0.91	62	236.0	205.0	11	177.1	21	ESI (+)
Dimethomorph	4.06	127	388.0	301.0	33	273.0	42	ESI (+)
Diniconazole	4.99	85	326.0	70.0	60	159.0	45	ESI (+)
Dodine	3.87	132	228.0	57.0	37	71.0	29	ESI (+)
Epoxiconazole	4.49	90	330.0	121.1	22	141.1	23	ESI (+)
Ethirimol	0.48	100	210.0	140.0	28	98.0	34	ESI (+)
Ethofenprox	8.35	60	394.1	177.1	20	359.2	15	ESI (+)
Ethoprophos	4.43	65	243.0	131.0	27	215.0	16	ESI (+)
Fenamidone	4.45	75	312.0	236.0	21	92.1	40	ESI (+)
Fenarimol	4.24	110	331.0	268.0	32	259.3	35	ESI (+)
Fenbuconazole	4.86	90	337.0	125.0	50	70.0	50	ESI (+)
Fenhexamid	4.52	100	302.0	97.0	30	55.0	60	ESI (+)
Fenitrothion	4.99	86	278.0	125.0	26	109.0	21	ESI (+)
Fenpropathrin	6.92	80	350.0	125.0	21	97.1	44	ESI (+)
Fenpropimorph	3.06	150	304.0	147.0	39	130.0	35	ESI (+)
Fenpyroximate	7.03	110	422.0	366.0	21	215.2	34	ESI (+)
Fenthion	5.59	80	279.0	247.0	17	169.1	23	ESI (+)
Fludioxonil	4.35	141,7	246.6	125.9	37	179.9	38	ESI (-)
Flusilazole	4.77	97	316.0	247.0	25	165.0	39	ESI (+)
Flutriafol	3.09	80	302.0	70.0	55	123.0	35	ESI (+)
Fosthiazate	2.99	62	284.0	228.0	13	104.0	32	ESI (+)
Hexaconazole	4.77	95	314.0	70.0	55	159.1	45	ESI (+)
Hexythiazox	6.94	51	353.0	228.0	19	271.0	20	ESI (+)
Imazalil	2.29	110	297.0	159.0	29	201.0	25	ESI (+)
Imidacloprid	0.84	65	256.0	209.0	22	175.0	27	ESI (+)
Iprodione	4.90	80	330.0	245.0	21	288.0	25	ESI (+)
Iprovalicarb	4.33	60	321.1	119.0	30	203.1	12	ESI (+)
Kresoxim-methyl	5.41	64	314.0	267.0	10	282.1	11	ESI (+)
Linuron-d_6 (I.S.)	4.05	74	255.0	160.0	26	185.0	24	ESI (+)
Malathion	4.98	75	331.0	127.0	16	285.0	10	ESI (+)
Malathion-d_{10} (I.S.)	4.86	76	341.0	132.0	19	100.0	37	ESI (+)
Mandipropamid	4.67	80	412.0	328.0	20	356.0	15	ESI (+)
Mepanipyrin	4.59	160	224.0	106.0	33	130.9	48	ESI (+)
Metalaxyl	3.24	62	280.2	220.0	19	191.9	32	ESI (+)
Metconazole	4.90	90	320.0	70.0	65	125.0	60	ESI (+)
Methamidophos	0.42	71	142.0	94.0	20	125.0	19	ESI (+)
Methidathion	4.06	55	303.0	145.0	14	85.0	28	ESI (+)
Methiocarb	3.99	60	226.0	169.0	13	121.0	25	ESI (+)
Methomyl	0.51	37	163.0	106.0	14	88.1	12	ESI (+)
Methoxyfenozide	4.81	50	369.0	313.2	11	149.1	28	ESI (+)
Myclobutanil	4.47	86	289.0	70.0	52	125.0	45	ESI (+)
Oxadixyl	2.14	67	279.0	219.2	14	102.0	14	ESI (+)
Oxydemeton-methyl	0.46	48	247.0	169.0	18	105.0	17	ESI (+)
Paclobutrazole	3.91	90	294.0	70.0	50	125.0	55	ESI (+)
Parathion	5.59	60	292.0	236.0	20	264.1	13	ESI (+)
Parathion-Methyl	4.58	70	264.0	232.0	23	125.0	23	ESI (+)
Penconazole	4.78	77	284.0	70.0	42	159.0	45	ESI (+)
Pencycuron	5.90	95	329.0	125.0	55	218.0	22	ESI (+)
Pendimethalin	6.89	40	282.0	212.1	16	194.0	26	ESI (+)
Phenthoate	5.65	66	321.0	163.0	15	247.0	15	ESI (+)
Phosalone	6.04	75	368.0	182.0	23	322.0	14	ESI (+)
Phosmet	4.44	62	318.0	160.0	21	133.0	63	ESI (+)

Table 1 (*continued*)

Compound	t_R (min)	DP (V)	Precursor ion (*m/z*)	Product ion (*m/z*)—SRM1	CE 1 (V)	Product ion (*m/z*) —SRM2	CE 2 (V)	Ionization mode
Phoxim	6.01	65	299.0	129.0	16	153.1	9	ESI (+)
Pirimicarb	0.52	70	239.0	182.1	21	72.1	39	ESI (+)
Pirimiphos-methyl	5.28	110	306.0	164.1	30	108.1	43	ESI (+)
Prochloraz	3.87	53	376.0	308.0	16	266.0	23	ESI (+)
Procimidone	5.00	80	283.9	256.0	25	228.0	30	ESI (+)
Profenofos	6.15	66	373.0	303.0	26	344.8	18	ESI (+)
Propargite	7.34	60	368.0	231.0	15	175.0	21	ESI (+)
Propiconazole	5.01	100	342.0	159.0	41	69.0	35	ESI (+)
Propoxur	2.48	50	210.0	168.0	11	111.1	20	ESI (+)
Propyzamide	4.51	70	256.0	190.0	21	173.0	33	ESI (+)
Prothiofos	7.92	80	344.8	241.0	25	269.0	16	ESI (+)
Pyraclostrobin	5.79	64	388.0	194.0	16	164.0	24	ESI (+)
Pyrethrins	7.32	62	329.0	161.0	13	143.0	23	ESI (+)
Pyridaben	7.57	90	365.1	309.0	19	147.0	35	ESI (+)
Pyrimethanil	2.04	135	200.0	107.1	32	168.1	40	ESI (+)
Pyriproxyfen	6.71	70	322.0	96.0	20	227.0	20	ESI (+)
Quinoxyfen	5.99	120	308.0	197.0	44	272.0	40	ESI (+)
Rotenone	5.04	120	395.0	213.0	35	192.0	36	ESI (+)
Spinosyn A	4.03	147	732.5	142.0	34	98.0	100	ESI (+)
Spinosyn D	4.27	154	746.5	142.0	36	98.0	97	ESI (+)
Tebuconazole	4.63	96	308.0	70.1	51	125.1	53	ESI (+)
Tebufenpyrad	6.23	110	334.0	145.0	35	117.0	60	ESI (+)
Teflubenzuron	6.07	46,6	378.9	338.8	12	358.9	11	ESI (-)
Tetraconazole	4.68	100	372.0	159.0	43	70.0	65	ESI (+)
Thiabendazole	0.46	142	202.0	174.9	37	131.1	44	ESI (+)
Thiacloprid	1.77	73	253.1	126.0	27	186.0	19	ESI (+)
Thiametoxam	0.55	58	291.9	211.0	17	131.9	36	ESI (+)
Thiodicarb	2.81	63	355.0	88.0	27	108.0	21	ESI (+)
Thiophanate-methyl	2.5	75	343.0	151.0	27	311.0	16	ESI (+)
Tolclofos-methyl	5.92	84	301.0	125.0	26	269.0	22	ESI (+)
TPP (I.S.)	5.62	106	326.8	77.0	63	152.0	53	ESI (+)
Triadimefon	4.49	70	294.0	197.0	22	225.1	18	ESI (+)
Trifloxistrobin	6.32	81	409.0	186.0	26	206.0	20	ESI (+)
Triflumuron	5.59	68	359.0	156.0	21	139.0	50	ESI (+)
Triticonazole	4.06	80	318.0	70.0	55	125.0	50	ESI (+)
Vamidothion	0.55	61	288.0	146.0	19	118.0	32	ESI (+)
Zoxamide	5.64	107	336.0	187.0	31	204.0	23	ESI (+)

I.S.: Internal standard.

For the correct identification and quantification of the pesticides the criteria from the EU guideline were adopted. The SRM transition with the best signal-to-noise ratio (SRM1) was used as quantifier transition and a second and more specific transition (SRM2) was used for identification. Moreover, was required the retention time coincidence with the standard (matrix-matched standard, with tolerance of $\pm$ 0.2 min), the real acquisition of two monitored SRM transitions and the compliance of the SRM ratio (ratio between SRM2/SRM1), when compared with calibration standard at comparable concentrations and measured under the same conditions [3]. The optimized parameters from the μLC–MS/MS acquisition method can be seen in Table 1.

2.5. Samples

Fifty-one fruit jam samples from eight different commercial brands were purchased in local markets of Santa Maria (South of Brazil), $n=34$ and Almería (South-eastern of Spain), $n=17$. The samples consisted of jams from five different fruits types, strawberry 300 g of slurry of each sample by the homogenization of 200 g of fruit jam with 100 g of ultra-purified water, in a Polytron-PT 10–35 (Switzerland) homogenizer during 2 min at 3000 rpm. The slurry portions were stored in a freezer at -20 °C until the analysis.

2.6. Extraction procedure

For recovery studies, the samples were spiked with the studied pesticides before the QuEChERS extraction procedure. Some samples obtained from the local markets were analyzed in order to provide a blank sample for the validation. An amount of 10 g of slurry (corresponding to 6.7 g of fruit jam) was weighed in a 50 mL PTFE centrifuge tube. The blank slurries of fruit jam were spiked with a volume of 60 or 300 μL of a mixture standard solution containing 1000 μg L^{-1} of each pesticide in order to provide spike concentrations of 9 and 45 μg kg^{-1}, respectively, with five replicates at each concentration. For the extraction step, a volume of 10 mL of acetonitrile containing the procedure internal standards (I.S.), diclorvos-d_6, malathion-d_{10} and triphenyl phosphate (TPP) at 50 μg L^{-1}, was added to the tubes and the samples were shaken in an automatic axial extractor (AGYTAX®, Cirta Lab. S.L., Spain) for 4 min. Afterwards, 4 g of magnesium sulfate, 1 g of sodium chloride, 1 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogencitrate-sesquihydrate were added and the samples were again shaken in the automatic axial extractor for 4 min. The extracts were then centrifuged (3700 rpm) for 5 min and 5 mL were transferred to a 15 mL PTFE centrifuge tube containing 750 mg of magnesium sulfate, 125 mg of PSA and 125 mg of C_{18}, for clean-up. The tubes were shaken in a vortex for 30 s and centrifuged again (3700 rpm) for further 5 min. Hereafter, 4 mL of the extracts were transferred to a vial and acidified with 50 μL of formic acid (5% in acetonitrile). Before the μLC–MS/MS injection the extracts were diluted 30-fold with a mixture of acetonitrile/water (1:9), this corresponded to the injection of just 0.022 g of sample per milliliter of diluted extract.

As already demonstrated in previous works, dilution of extracts is a good way to eliminate matrix effects [21,22]. Even when they are commodity dependent it was demonstrated that dilution factors of 25–40 can eliminate the majority of them [22].

Thereby, to verify the correct execution of the dilution step, linuron-d_6 was added to acetonitrile extracts at the concentration of 10 μg L^{-1}, before the dilution. That was done by pipetting 96 μL of the acidified acetonitrile extract to an 1.5 mL vial and adding 4 μL of Linuron-d_6 solution (250 μg L^{-1}), providing a concentration of 10 μg L^{-1}. These solutions were used for the following 30-fold dilution step, which was performed in another 1.5 mL vial by transferring 570 μL of acetonitrile/water (1:9), 20 μL of the acetonitrile solution with linuron-d_6 at 10 μg L^{-1} (prepared in the previous step) and 10 μL of dimethoate-d^6 (60 μg L^{-1}), as injection I.S., providing a concentration of 1 μg L^{-1}. These vials were taken for injection in the μLC–MS/MS equipment.

2.7. Method validation

All the 107 target pesticides of this study and 5 I.S., were evaluated in one single chromatographic run by μLC–MS/MS in the positive and negative ESI–sMRM mode.

2.7.1. Calibration curves, linearity and matrix effect

The calibration curves were constructed based on peak areas obtained from injection of standard solutions prepared in blank grape jam extracts and in neat acetonitrile, at the following concentrations: 6, 30, 60, 100, 200, 300 and 400 μg L^{-1} (corresponding to pesticide residue concentrations of 9, 45, 90, 150, 300, 450 and 600 μg kg^{-1} in the grape jam samples). Because of the high sensitivity of the μLC–MS/MS equipment and to minimize the matrix effect, these solutions were diluted 30-fold before injection providing the concentration of 0.2, 1.0, 2.0, 3.3, 6.6, 10.0 and 13.3 μg L^{-1} (corresponding to 0.3; 1.5; 3.0; 5.0; 10.0; 15.0; 20.0 in blank grape jam extract).

The linearity of the calibration curves was assessed by calculating the determination coefficients (r^2). The linear range was also determined for each analyzed pesticide.

The matrix effect was calculated comparing the slope of the calibration curves in matrix (grape jam extract) and acetonitrile using the following equation:

$$\text{Matrix effect (\%)} = \left[\left(\frac{\text{slope curve, standard in matrix}}{\text{slope curve, standard in acetonitrile}}\right) - 1\right] x\ 100$$

2.7.2. Accuracy (trueness and precision)

Accuracy is the closeness of agreement between a test result and the true or the accepted reference value. When applied to a set of test results, it involves a combination of random error (estimated as precision) and a common systematic error (trueness or bias). Precision is defined as the closeness of agreement between independent analytical results obtained by applying the experimental procedure under stipulated conditions. The smaller the random part of the experimental error which affects the results, the more precise the procedure. A measure of precision (or imprecision) is the standard deviation [3].

The accuracy (trueness and precision) of the method was evaluated through recovery experiments by spiking pesticides to a blank grape jam slurry, at two different concentrations (9 and 45 μg kg^{-1}), with five replicates at each concentration ($n=5$). The spiking procedure was performed by adding the standard mixture solution containing the pesticides to the jam slurry. The average peak areas were used to calculate recoveries (%) and the RSD% at the different spike concentrations.

2.7.3. Reporting limit (RL) or limit of quantification (LOQ)

According to SANCO [3] the RL is the lowest level at which residues will be reported as absolute numbers and it is equal to, or higher than the LOQ. In this study it was based on the accuracy and precision data, obtained via the recovery determinations and was defined as the lowest validated spike concentration meeting the requirements of an average recovery within the range 70–120% and an RSD ≤ 20%.

3. Results and discussion

3.1. Accuracy (recovery), precision, RL and selectivity

The method was assessed for accuracy and precision by the analysis of spiked grape jam samples at two concentrations, 9 and 45 μg kg^{-1}, with five replicates at each concentration. The individual recovery results are reported in Table 2. For the spike concentrations of 9 and 45 μg kg^{-1}, the number of compounds that fulfilled the requirements for validation (recoveries 70–120% and RSD ≤ 20%) was 71 and 94, respectively.

Dodine was detected at the concentration of 45 μg kg^{-1}, in the calibration standards in matrix and in acetonitrile but was not detected at the recovery samples at the same concentration. That can be explained by its specific properties like molecular structure, (*n*-dodecylguanidine acetate) and its relatively high solubility in water at acidic pH (around 5) so it could have been not completely extracted from the fruit jam slurry [23].

Mepanipyrin first transition was detected at both concentrations studied but could not be confirmed due the low signal of its second transition. Thiodicarb was not detected at the both spike concentrations studied and even at concentrations higher than 45 μg kg^{-1}. This pesticide belongs to class of the carbamate pesticides and has methomyl as metabolite [24]. Methomyl was not in the standard pesticide mixture solution (1000 μg L^{-1}), but was detected in the recovery samples with recoveries of 97% (RSD=21%) at the spike concentration of 9 μg kg^{-1} and rec. of 91% (RSD=10%) at the concentration of 45 μg kg^{-1}, it is a consequence of thiodicarb degradation to methomyl.

The recovery (%) and RSD% data were also used for the establishment of the RL which are also reported in Table 2. According to this table, 66% and 26% of the analytes had the RL established at 9 and 45 μg kg^{-1}, respectively. Three percent were not detected and 5% had recoveries outside the range of 70–120% and/or RSD > 20% at both concentrations. Thus, in total 92% of the compounds satisfied the validation requirements at the studied levels.

In order to maintain the selectivity of the method and correct identification of the pesticides, besides the correct relative intensities of the SRM transitions of each pesticide, the retention times of the analytes are also very important and have to be reproducible [25,26]. Clean-up and/or dilution steps reduce matrix interferences also resulting in improved selectivity and reduce contamination of the instrument systems leading to improved robustness [3].

In this method the selectivity can be seen in Fig. 1, where are shown the overlapped total ion chromatograms of standard solutions in acetonitrile and grape jam extract and a blank grape jam extract. But the selectivity is illustrated even better in Fig. 2, where are shown the SRM transitions of carbendazim, pyrimethanil and difenoconazole, that were detected in positive strawberry and grape jam samples.

Finally, to ensure the correct identification of all analytes, in the case of occurrence of signals in blank matrix extracts in the range of ± 0.2 min of the pesticide retention time, it was ensured that it did not exceed the expected analyte peak intensity at 30% at the LOQ.

3.2. Calibration curves, linearity and linear range

The method showed to be linear ($r^2 \geq 0.99$) in the range of 6–400 μg L^{-1} (corresponding to the range of 9–600 μg kg^{-1} in fruit jam) for the majority of the pesticides. Between the 99 fully validated compounds just fenitrothion had an $r^2 < 0.99$ (0.98)

Table 2
Average recoveries (%), RSD (%) ($n=5$), matrix effect (M.E.%) and reporting limit (RL) calculated from the standard solutions prepared in blank grape jam extract and in acetonitrile. RL is referred to the concentration in the sample. Detection was performed by μLC–MS/MS.

Compound	Spike concentration				M.E. (%)	RL (μg kg^{-1})
	9 μg kg^{-1}		45 μg kg^{-1}			
	Average recovery (%)	RSD (%)	Average recovery (%)	RSD (%)		
Acephate	142	20	111	6	−60	45
Acetamiprid	96	4	92	4	−4	9
Azinphos-methyl	n.d.	n.d.	109	39	n.f.r.	n.f.r.
Azoxystrobin	82	16	117	41	−1	9
Bitertanol	111	18	90	15	4	9
Boscalid	n.d.	n.d.	118	7	−4	45
Bromuconazole	n.d.	n.d.	97	31	n.f.r.	n.f.r.
Bupirimate	104	3	91	3	1	9
Buprofezin	152	14	89	13	10	45
Carbaryl	97	6	92	4	−8	9
Carbendazim	75	6	61	4	−25	9
Carbofuran	128	6	109	4	−10	45
Chlorpyrifos	111	6	89	3	−1	9
Chlorpyrifos methyl	140	20	89	10	4	45
Cyproconazole	159	8	92	7	−4	45
Cyprodinil	n.d.	n.d.	95	5	18	45
Diazinon	n.d.	n.d.	36	5	n.f.r.	n.f.r.
Diclorvos	n.d.	n.d.	104	9	−7	45
Dicrotophos	84	9	82	5	−12	9
Diethofencarb	158	32	91	16	−5	45
Difenoconazole	104	6	90	4	0	9
Dimethoate	103	5	91	4	−7	9
Dimethomorph	116	8	91	2	−8	9
Diniconazole	105	4	88	5	−1	9
Dodine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Epoxiconazole	134	19	97	6	4	45
Ethirimol	94	10	65	7	−6	9
Ethofenprox	120	6	113	4	−33	9
Ethoprophos	n.d.	n.d.	42	10	n.f.r.	n.f.r.
Fenamidone	107	15	91	5	−3	9
Fenarimol	n.d.	n.d.	97	10	−8	45
Fenbuconazole	107	6	94	5	2	9
Fenhexamid	133	8	77	16	5	45
Fenitrothion	n.d.	n.d.	98	11	9	45
Fenpropathrin	110	12	89	6	−3	9
Fenpropimorph	91	9	81	4	14	9
Fenpyroximate	118	7	95	5	−19	9
Fenthion	110	11	88	3	−1	9
Fludioxonil	n.d.	n.d.	100	13	0	45
Flusilazole	97	7	89	3	0	9
Flutriafol	104	6	89	3	−5	9
Fosthiazate	104	5	92	4	−7	9
Hexaconazole	102	5	86	5	0	9
Hexythiazox	113	4	89	1	0	9
Imazalil	104	11	83	3	8	9
Imidacloprid	106	5	87	6	−4	9
Iprodione	n.d	n.d.	102	7	16	45
Iprovalicarb	108	31	88	11	−5	45
Kresoxim-methyl	101	6	90	5	−4	9
Malathion	108	5	89	3	−14	9
Mandipropamid	94	13	92	3	−4	9
Mepanipyrin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Metalaxyl	107	5	92	4	0	9
Metconazole	123	10	89	8	1	45
Methamidophos	n.d.	n.d.	75	6	−59	45
Methidathion	107	13	82	9	−7	9
Methiocarb	111	5	91	4	−1	9
Methomyl	97	21	91	10	−16	9
Methoxyfenozide	115	9	92	6	-5	9
Myclobutanil	92	5	88	8	3	9
Oxadixyl	117	21	89	14	−6	9
Oxydemeton-methyl	92	12	78	6	−21	9
Paclobutrazole	106	7	98	18	−2	9
Parathion	82	11	90	7	16	9
Parathion-Methyl	n.d.	n.d.	100	19	18	45
Penconazole	113	6	89	5	2	9
Pencycuron	121	11	88	5	−6	45
Pendimethalin	110	5	88	3	−3	9
Phenthoate	117	7	93	3	2	9

Table 2 (*continued*)

Compound	Spike concentration				M.E. (%)	RL (μg kg^{-1})
	9 μg kg^{-1}		45 μg kg^{-1}			
	Average recovery (%)	RSD (%)	Average recovery (%)	RSD (%)		
Phosalone	118	20	85	11	1	9
Phosmet	107	26	89	9	−7	45
Phoxim	n.d.	n.d.	96	2	−5	45
Pirimicarb	108	8	90	5	−7	9
Pirimiphos-methyl	104	5	88	4	1	9
Prochloraz	104	7	87	5	0	9
Procimidone	n.d.	n.d.	98	6	10	45
Profenofos	118	35	93	4	−2	45
Propargite	115	6	96	3	−16	9
Propiconazole	107	7	88	3	1	9
Propoxur	113	6	93	3	−7	9
Propyzamide	114	15	90	9	−4	9
Prothiofos	114	3	90	3	−23	9
Pyraclostrobin	110	8	93	3	1	9
Pyrethrins	108	3	95	3	−10	9
Pyridaben	119	4	102	4	−26	9
Pyrimethanil	101	10	87	6	−1	9
Pyriproxyfen	104	4	88	2	0	9
Quinoxyfen	99	10	87	3	2	9
Rotenone	105	4	89	4	−2	9
Spinosyn A	78	5	65	7	38	9
Spinosyn D	n.d.	n.d.	62	15	n.f.r.	n.f.r.
Tebuconazole	107	7	93	7	−3	9
Tebufenpyrad	113	33	91	2	−5	45
Teflubenzuron	120	42	91	10	3	45
Tetraconazole	108	8	94	4	5	9
Thiabendazole	107	2	65	6	−14	9
Thiacloprid	96	4	90	6	54	9
Thiametoxam	91	7	91	5	−13	9
Thiodicarb	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Thiophanate-methyl	115	7	115	6	−2	9
Tolclofos-methyl	87	23	86	7	1	45
Triadimefon	102	9	87	6	−2	9
Trifloxistrobin	106	7	92	3	−2	9
Triflumuron	113	7	92	3	−6	9
Triticonazole	107	10	90	4	1	9
Vamidothion	98	3	92	4	-13	9
Zoxamide	n.d.	n.d.	96	2	0	45

n.f.r.: not fulfilling requirements for quantitative method (recovery: 70–120%, RSD ≤ 20%); n.d.: not detected.

considering the calibration curves prepared in blank grape jam extracts. For the calibration curves in acetonitrile just carbendazim and thiabendazole presented $r^2 < 0.99$, both had $r^2 = 0.98$.

3.3. Matrix effect

The matrix effect (%) was calculated by comparing the slope of the calibration cures in blank grape jam extract (matrix matched calibration standards) and in acetonitrile of each pesticide. In order to reduce the matrix effect, or matrix interference, a clean-up step was used during the extraction procedure and later the acetonitrile extract was diluted 30-fold before the μLC–MS/MS injection. The individual matrix effect can be seen in Table 2 and were under 20% for the most of the pesticides (92%). It was also observed that the matrix effect was negative (suppression) in the majority of the cases. In LC the negative matrix effect represents a loss of the analytical signal (ion suppression) due to alterations in the ionization efficiency [27,28]. Thus it is likely to use matrix matched calibration for the analytes that the matrix effect exceeds 20%.

3.4. Fruit jam samples analysis

As a part of this study were analyzed 51 samples of apricot, grape, peach, pineapple and strawberry jams belonging to eight different brands from Brazil and from Spain. To ensure the veracity of the results, even when the detected pesticides fulfilled the prerequisites of the correct retention time and ion ratio in comparison with the standards in matrix, the positive samples with pesticides at concentrations ≥ 20 μg kg^{-1} were re-analyzed by LC–QqQ–MS/MS, GC–QqQ–MS/MS and/or LC–Orbitrap–MS/MS in order to confirm the results (pesticide and concentrations) and when the detected pesticides did not fulfill all prerequisites in both systems they were not reported as positives.

As can be seen at Table 3, 80% of the samples were positive for at least one pesticide and in total were detected 42 pesticides. The most contaminated samples were the strawberry jam samples. In all the strawberry jam samples from Brazil was found difenoconazole (at concentrations up to 64 μg kg^{-1}), procimidone (conc. up to 1575 μg kg^{-1}) and thiophanate-methyl (conc. up to 959 μg kg^{-1}). In almost all these samples was also detected azoxystrobin (conc. from 10 to 151 μg kg^{-1}), carbendazim (conc. from 20 to 221 μg kg^{-1}), fenpyroximate (conc. up to 18 μg kg^{-1}), imidacloprid (conc. from 10 to 67 μg kg^{-1}), iprodione (conc. up to 654 μg kg^{-1}) and pyrimethanil (conc. up to 202 μg kg^{-1}). In the strawberry jams from Spain, penconazole and spinosyn A were the top detected analytes, but at concentrations lower that the RL and azoxystrobin was found at the highest concentration 33 μg kg^{-1} in one sample.

In grape jams from Brazil, pyrimethanil was detected most frequently and it was also the one detected at the highest concentration (81 μg kg^{-1}). In pineapple jam, also samples from

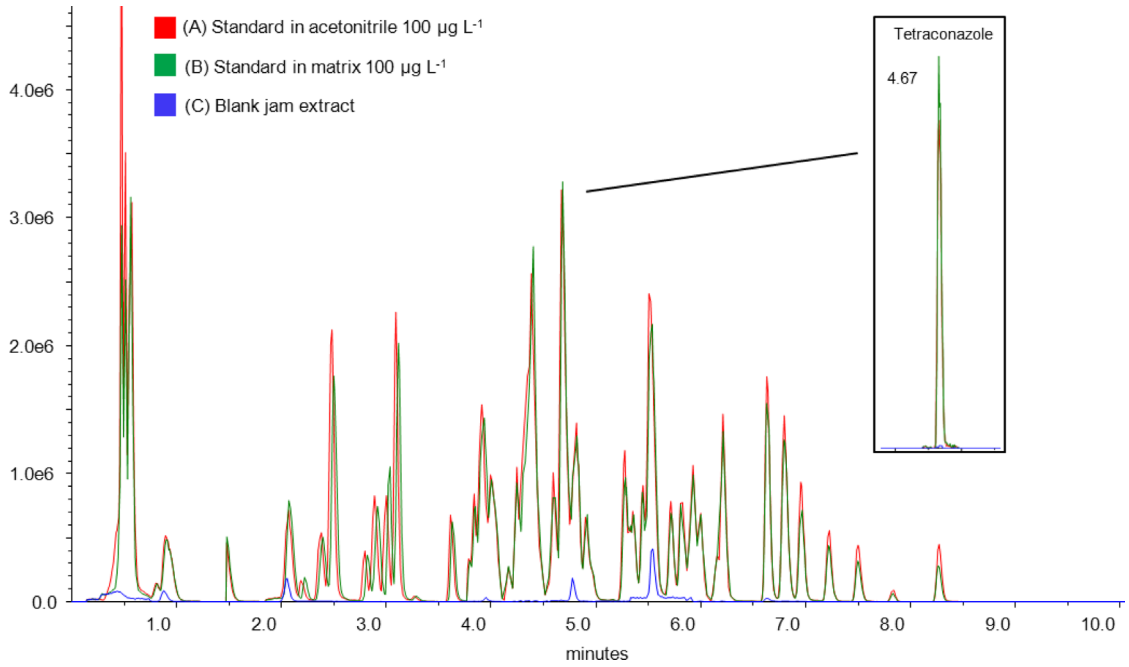

Fig. 1. Total ion chromatograms obtained by analysis via µLC–MS/MS of (A) standard pesticide mixture solution in acetonitrile at 100 µg L^{-1}, (B) standard pesticide mixture solution in blank grape jam extract at 100 µg L^{-1} (150 µg kg^{-1}) and (C) blank grape jam extract, showing the small matrix effect (suppression) of the jam grape extract.

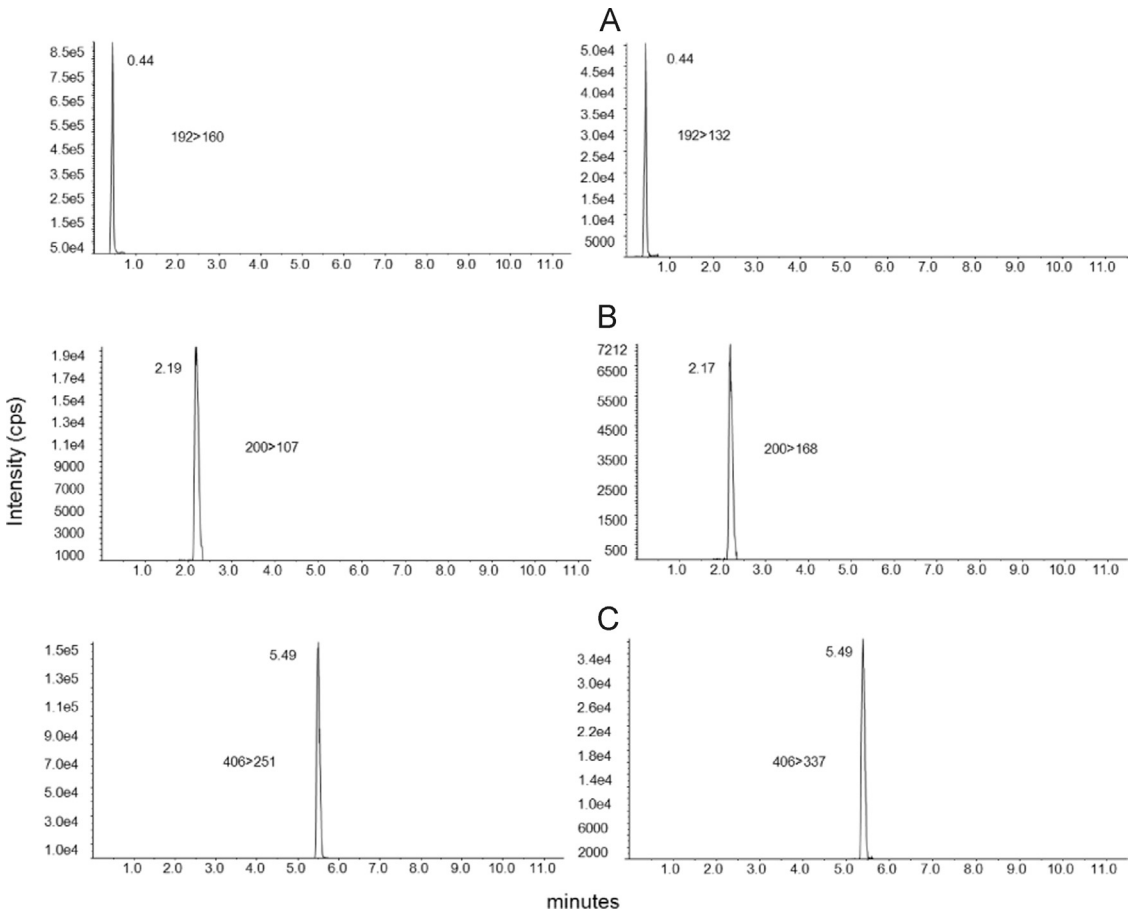

Fig. 2. SRM transitions of positive samples obtained by analysis via µLC–MS/MS for carbendazim at 221 µg kg^{-1} in (A) strawberry jam sample, (B) pyrimethanil at 62 µg kg^{-1} in a grape jam sample and (C) difenoconazole at 64 µg kg^{-1} in a strawberry jam sample.

Brazil, carbendazim was found more frequently and at the highest concentration (32 µg kg^{-1}).

The peach and apricot jams (from Spain) presented less pesticide residues when compared to the other samples. Apricot jam presented most frequently imidacloprid at low concentrations from 9 to 11 µg kg^{-1} and myclobutanil (conc. 12 and 13 µg kg^{-1}). In peach jam was detected more frequently chlorpyrifos but at concentrations lower than the RL.

Table 3
Pesticides more frequently detected in the fruit jams, percentage of positive samples (where at least one pesticide was detected) and samples with pesticides at concentrations ≥ 10 μg kg^{-1} according to fruit jam and country of origin.

Pesticide	Positive samples (%)						Total (n=51)
	Strawberry jam		Grape jam	Pineapple jam	Peach jam	Apricot jam	
	Brazil (n=12)	Spain (n=5)	Brazil (n=12)	Brazil (n=10)	Spain (n=8)	Spain (n=4)	
Carbendazim	75	0	25	30	13	0	31
Difenoconazole	100	0	8	0	13	0	27
Imidacloprid	50	40	25	0	0	75	27
Pyrimethanil	75	0	33	0	0	0	25
Fenpiroxymate	75	60	0	0	0	0	24
Procimidone	100	0	0	0	0	0	24
Thiophanate-methyl	100	0	0	0	0	0	24
Iprodione	75	0	8	0	0	0	20
Tebuconazole	42	0	8	10	13	25	18
Azoxystrobin	58	20	0	0	0	0	16
Fenhexamid	25	20	17	20	0	0	16
Metalaxyl	50	0	8	0	0	0	14
Spinosyn A	25	80	0	0	0	0	14
Penconazole	0	80	0	20	0	0	12
Chlorpyrifos	17	0	0	0	25	0	8
Kresoxim-methyl	33	0	0	0	0	0	8
Myclobutanil	0	40	0	0	0	50	8
Boscalid	8	40	0	0	0	0	6
Propargite	25	0	0	0	0	0	6
Thiametoxam	25	0	0	0	0	0	6
Total	100	100	67	70	75	75	80
≥ 10 μg kg^{-1}	100	60	42	20	13	75	51

As the fruit jam can be prepare according to different industrial procedures, differing in to amount of fruits (g of fruit/g of jam), water, sugar as well as different cooking times and ways and presence or absence of additives, it is difficult to know exactly the influence of each factor on the pesticide residue concentration in the final fruit jam. But it is clearly evident that the pesticides, independent from where they come from e.g. fruits, water or sugar, remain present in the final fruit jam and contribute for the pesticide daily intake of human beings inferring that these products should be controlled for pesticides residues.

Either in Brazil and in the EU there are no MRL established for this type of commodities, but as the presence of pesticides was demonstrated by this study the need of control the occurrence of residues in these products should be considered.

4. Conclusions

In this study a very sensitive μLC–MS/MS multi-residue method was developed and validated for the determination of 99 pesticides in fruit jams. To minimize the matrix effect (or matrix interference) and increase the selectivity of the method, a clean-up and a dilution step were applied to the fruit jam extracts, before μLC-MS/MS analysis. The method presented good accuracy (recoveries%) and precision (RSD%) for 92% of the pesticides studied. Furthermore, the method had also a wide linear range (from 9 to 600 μg kg^{-1}), good linearity ($r^2 \geq 0.99$) and low RL (9 μg kg^{-1}) for the majority of the analytes evaluated. Fifty-one jam samples of apricot, grape, peach, pineapple and strawberry (from Brazil and from Spain) were analyzed in order to evaluate the presence of pesticide residues. In total 80% of the samples were positive for at least one pesticide. The most contaminated samples of this study were the strawberry jams with 100% of positive samples and among them, the samples from Brazil were the ones with the largest number of detected pesticides and with the highest concentrations, e.g. procimidone at 1575 μg kg^{-1} in one sample. The pesticide more frequently detected was carbendazim, present in 31% of the samples.

According to the results presented in this study, is clearly evident the occurrence of pesticide residues in fruit jams. Thus the control of pesticide residues in these food commodities should be applied because certainly fruit jams contribute for pesticide daily intake of human beings.

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